

FavorPrepTM
96-well Gel/PCR Clean-Up Kit

User Manual

**Cat. No.: FAPKE 001
FAPKE 002**

**For Research Use Only
v.1211-1**

Introduction

FavorPrep™ 96-well GEL/ PCR Clean Up kit is designed for rapid purification of fragment DNA from agarose gel , PCR or other enzymatic reaction. The procedure uses chaotropic salt to dissolve agarose and denature enzyme. With the suitable binding condition provided by this system, the DNA in the sample mixture binds to glass fiber matrix in the 96-well DNA Binding Plate. The contaminants are washed with an ethanol-contained wash buffer and finally the purified DNA is eluted by low salt Elution buffer or water. The protocol does not require phenol extraction and alcohol precipitation. The entire procedure can be done within 30~40 minutes and the purified DNA is ready for restriction digestion, ligation, labeling, PCR, and sequencing reaction.

Quality Control

The quality of 96-Well Gel/PCR Kit is tested on a lot-to-lot basis. The purified DNA fragment is checked by agarose gel analysis and quantified with spectrophotometer.

Kit Content

	FAPKE001	FAPKE002
Bind Buffer D1	130 ml	330 ml
Wash Buffer (concentrated)*	50 ml	50 ml x 3
Elution Buffer	30 ml	60 ml
96-Well DNA Binding plate	4 pcs	10 pcs
96-Well PCR plate	4 pcs	10 pcs
Adhesive Film	5 pcs	12 pcs

* Add 200 ml of ethanol (96~100%) to each Wash Buffer when first use.

Specification

Sample Size: up to 50 μ l PCR or other enzymatic reaction mixture
Up to 100 mg agarose gel slice

Binding Capacity: up to 20 μ g/ well

DNA Size range: 70 bp~12Kb

Operation: centrifuge/ vacuum manifold

Handling Time: about 30 minutes for PCR clean up
about 40 minutes for gel DNA extraction

Recovery: 90~95 % PCR clean up

70~85 % for gel DNA extraction

Downstream application: Fluorescent or radioactive sequencing, Restriction digestion, Library screening, Ligation, Labeling, Transformation

Important Note

1. Buffer provided in this kit contain irritants. Wear gloves and lab coat when handling these buffers.
2. When excising the agarose gel, remove the extra gel to minimize the size of the gel.
3. Preheat a water bath to 50 °C for Gel DNA Extraction Protocol.
4. Add 200 ml of ethanol (96~100%) to each Wash Buffer before first open.

Gel DNA Extraction Protocol (Centrifuge)

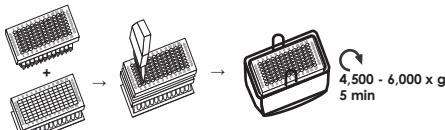
Please Read Important Notes Before Starting The Following Steps.

Step 1

- Transfer up to 100 mg of agarose gel (containing relevant DNA fragment) to each well of a clean 96-Well 2 ml Plate (not provided).
- Add 300 μ l of Binding Buffer D1 to each well, sealing with a adhesive film. Incubate at 50 °C for 10~15 minutes until the gel slice dissolved completely. During the incubation, briefly shake the incubated plate for every 5 minutes to make the sample mixture mix well with Binding Buffer D1.

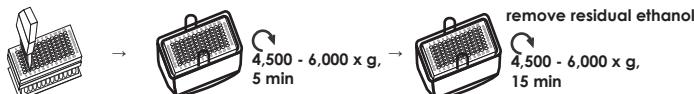
Step 2: DNA Binding

- Place a 96-Well DNA Binding Plate on top of a 96-Well 2 ml Plate (not provided).
- Transfer the sample mixture from step 1 to the 96-Well DNA Binding Plate.
- Place the assembly plates in a rotor bucket and centrifuge at 4,500-6,000 x g for 5 min.
- Discard the flow-through and return the 96-Well DNA Binding Plate to the 96-Well 2 ml Plate.



STEP 3: Washing

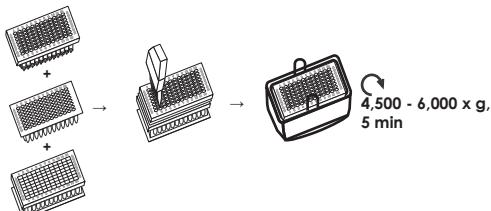
- Add 650 μ l of Wash Buffer (ethanol added) to each well of the 96-Well DNA Binding Plate.
- Place the assembly plates in a rotor bucket and centrifuge at 4,500 – 6,000 x g for 5 min.
- Discard the flow-through and return the 96-Well DNA Binding Plate back to the 96-Well 2 ml Plate.
- Place the assembly plates in a rotor bucket and centrifuge at 4,500 – 6,000 xg for an additional 15 minutes to remove residual ethanol.



STEP 4

DNA Elution

- Place a clean 96-well PCR Plate (provided) on top of a clean 96-Well 2 ml Plate (not provided). And place the 96-Well DNA Binding Plate on top of the clean 96-well PCR plate. (top: 96-well DNA Binding Plate, middle: 96-well PCR Plate, bottom: 96-Well 2 ml plate)
- Add 50 ~ 75 μ l of Elution Buffer or ddH₂O (pH 8.0~8.5) on the membrane center of the 96-Well DNA Binding Plate. Stand for 3 minutes until Elution Buffer or ddH₂O has been absorbed by the membrane completely.
- Place the combined plates in a rotor bucket and centrifuge for 5 min at 4,500 – 6,000 x g for 5 min to elute purified DNA.



Gel DNA Extraction Protocol (Vacuum)

Please Read Important Notes Before Starting The Following Steps.

Step 1

- Transfer up to 100 mg of agarose gel (containing relevant DNA fragment) to each well of a clean 96-Well 2 ml Plate (not provided).
- Add 300 μ l of Binding Buffer D1 to each well, sealing with a adhesive film. Incubate at 50 °C for 10–15 minutes until the gel slice dissolved completely. During the incubation, briefly shake the incubated plate for every 5 minutes to make the sample mixture mix well with Binding Buffer D1.

Step 2: DNA Binding

- Place a 96-Well DNA Binding Plate on top of the vacuum manifold.
- Transfer the sample mixture from step 1 to each well of the 96-Well DNA Binding Plate.
- Apply vacuum at 10 inches Hg for 3 minutes until wells have emptied.



Step 3: Washing

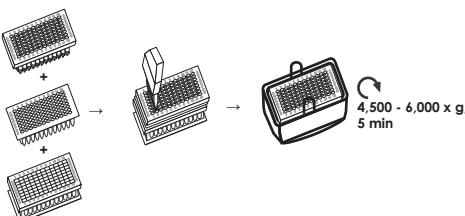
- Add 650 μ l of Wash Buffer (ethanol added) to each well of the 96-Well DNA Binding Plate.
- Apply vacuum at 10 inches Hg for 5 minutes until wells have emptied.
- Apply vacuum at 10 inches Hg for an additional 10 min (or incubate at 60 °C for 10 min) to remove residual ethanol.



STEP 4

DNA Elution

- Place a clean 96-well PCR Plate (provided) on top of a clean 96-Well 2 ml Plate (not provided). And place the 96-Well DNA Binding Plate on top of the clean 96-Well PCR plate. (top: 96-well DNA Binding Plate, middle: 96-well PCR Plate, bottom: 96-Well 2 ml plate)
- Add 50 ~ 75 μ l of Elution Buffer or ddH₂O (pH8.0~8.5) on the membrane center of the 96-Well DNA Binding Plate. Stand for 3 minutes until Elution Buffer or ddH₂O has been absorbed by the membrane completely.
- Place the combined plates in a rotor bucket and centrifuge for 5 min at 4,500 – 6,000 \times g for 5 min to elute purified DNA.



PCR Clean Up Protocol (Centrifuge)

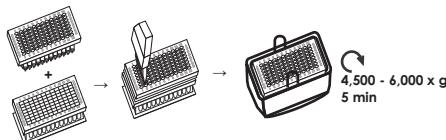
Please Read Important Notes Before Starting The Following Steps.

Step 1

- Transfer up to 50 μ l of PCR or enzymatic product to each well of a clean 96-Well 2 ml Plate. (not provided)
- Add 250 μ l of Binding Buffer D1 to each well. Mix well by Pipetting

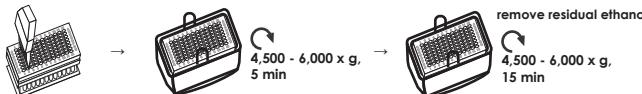
Step 2: DNA Binding

- Place a 96-Well DNA Binding Plate on top of a 96-Well 2 ml Plate (not provided).
- Transfer the sample mixture from step 1 to the 96-Well DNA Binding Plate.
- Place the assembly plates in a rotor bucket and centrifuge at 4,500-6,000 x g for 5 min.
- Discard the flow-through and return the 96-Well DNA Binding Plate to the 96-Well 2 ml Plate.



STEP 3: Washing

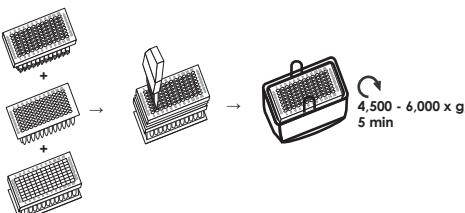
- Add 650 μ l of Wash Buffer (ethanol added) into each well of the 96-Well DNA Binding Plate.
- Place the assembly plates in a rotor bucket and centrifuge at 4,500 – 6,000 x g for 5 min.
- Discard the flow-through and return the 96-Well DNA Binding Plate back to the 96-Well 2 ml Plate.
- Place the assembly plates in a rotor bucket and centrifuge at 4,500 – 6,000 x g for an additional 15 minutes to remove residual ethanol.



STEP 4

DNA Elution

- Place a clean 96-well PCR Plate (provided) on top of a clean 96-Well 2 ml Plate (not provided). And place the 96-Well DNA Binding Plate on top of the clean 96-Well PCR plate. (top: 96-well DNA Binding Plate, middle: 96-well PCR Plate, bottom: 96-Well 2 ml plate)
- Add 50 ~ 75 μ l of Elution Buffer or ddH₂O (pH8.0~8.5) on the membrane center of the 96-Well DNA Binding Plate. Stand for 3 minutes until Elution Buffer or ddH₂O has been absorbed by the membrane completely.
- Place the combined plates in a rotor bucket and centrifuge for 5 min at 4,500 – 6,000 x g for 5 min to elute purified DNA.



PCR Clean Up Protocol (Vacuum)

Please Read Important Notes Before Starting The Following Steps.

Step 1

- Transfer up to 50 μ l of PCR or enzymatic product to each well of a clean 96-Well 2 ml Plate. (not provided)
- Add 250 μ l of Binding Buffer D1 to each well. Mix well by Pipetting.

Step 2: DNA Binding

- Place a 96-Well DNA Binding Plate on top of the vacuum manifold.
- Transfer the sample mixture from step 1 to each well of the 96-Well DNA Binding Plate.
- Apply vacuum at 10 inches Hg for 3 minutes until wells have emptied.



Step 3: Washing

- Add 650 μ l of Wash Buffer (ethanol added) to each well of the 96-Well DNA Binding Plate.
- Apply vacuum at 10 inches Hg for 5 minutes until wells have emptied.
- Apply vacuum at 10 inches Hg for an additional 10 min (or incubate at 60 $^{\circ}$ C for 10 min) to remove residual ethanol.



STEP 4

DNA Elution

- Place a clean 96-well PCR Plate (provided) on top of a clean 96-Well 2 ml Plate (not provided). And place the 96-Well DNA Binding Plate on top of the clean 96-Well PCR plate. (top: 96-well DNA Binding Plate, middle: 96-well PCR Plate, bottom: 96-Well 2 ml plate)
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